

Immunologic Benefits associated with Suppression of HIV Replication during Treatment for HIV-TB Co-infection

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ABSTRACT

Background: HIV-TB co-infection is associated with increased levels of immune activation, viral replication, CD4 T cell dysfunction and decline contributing to accelerated morbidity and mortality. Study objective was to determine if 6 months of ART given concurrently with TB therapy would produce sustained reductions in immune activation and viral load (VL), and improvements in CD4 T cell counts and function, when compared to TB treatment alone.

Methods: CD4 T cell counts, VL, markers of immune activation, and T cell effector function (interferon-gamma production/IFN-g), were measured among HIV-TB co-infected patients (CD4 T cell counts >350 cells/mL) at enrollment and every 3 months during 6 months of therapy and 6 months of observation in two arms: 38 patients treated with 6 month TB treatment (control); 38 patients treated with 6 month ART given concurrently with TB treatment (intervention).

Results: At enrollment, mean CD4 T cell counts (642.3 cells/mL ± 242 and 579.1 cells/mL ± 235) and VL (4.4 ± 0.78 log₁₀ copies/ml and 4.3 ± 1 log₁₀ copies/ml) were similar in control and intervention arms, respectively. Significant changes in CD4 T cell count were not observed in either arm during the study. VL in the intervention arm declined at 3 (2.1 ± 0.45 log₁₀ copies/ml, p<0.001) and 6 months (2.3 ± 0.77 log₁₀ copies/ml, p<0.0001) that was not sustained after ART was stopped. Within both arms, reductions in CD4/HLA-DR+/CD38+ and CD8/HLA-DR+/CD38+ were observed during 6 months of therapy and maintained through 6 month of observation (p<0.01). The intervention arm had lower levels of HLA-DR+/CD38+ CD8 T cells at 3 and 6 months of treatment when compared with the control arm (p<0.01). IFN-g production in response to PHA increased significantly in the intervention arm during the 6 months of therapy and was maintained through 6 month observation (p<0.01). Preliminary analysis of IFN-g production in response to MTB culture filtrate, MTB Ag85b, and HIV p24 antigens, did not demonstrate any significant differences or changes in either arm throughout the study.

Conclusions: In patients with HIV-TB co-infection and CD4 T cell counts >350 cells/mL, both TB treatment and concurrent HIV-TB treatment produce sustained decreases in immune activation, and this reduction is most pronounced in patients receiving ART while on therapy. Patients receiving concurrent HIV-TB treatment had sustained increases in global T cell effector function measured by IFN-g production in response to PHA.

INTRODUCTION

Tuberculosis remains a leading cause of death among HIV-infected individuals worldwide, with over half of untreated patients in Africa with AIDS having evidence of occult disseminated TB at death [1]. Although the development of TB in HIV-infected patients is associated with an increased risk of AIDS and death, optimal treatment strategies for co-infected patients remain controversial.

In addition to progressive CD4+ T cell decline, several other immune dysfunctions have been attributed to HIV infection such as enhanced T cell activation, decreased T cell proliferative responses to antigens, impaired T cell cytokine production, and alteration of T cell subsets. Although the pathogenesis of TB-HIV co-infection is not well understood, MTB infection is believed to provide an immunologic environment characterized by cytokine and chemokine irregularities permissive for enhanced HIV-replication and immune activation [2]. Immune activation is a strong predictor of disease progression in HIV-infection, and may contribute to the increased mortality associated with HIV-TB co-infection.

OBJECTIVE

Study objective was to determine if 6 months of ART (Trizivir: AZT, 3TC, ABC) given concurrently with standard TB therapy would produce sustained reductions in immune activation and viral load (VL), and improvements in CD4 and CD8 T cell counts, subsets, and function, when compared to TB treatment alone.

METHODS

A prospective cohort study, composed of participants within a larger phase 3 open-label randomized controlled clinical trial entitled "Randomized Clinical Trial of a 6-Month Punctuated Course of Antiretroviral Therapy in Ugandan HIV+ Adults with Pulmonary TB and CD4 >350 cells/mm³ (PART)". PART Participants (n=232) were followed for 24 months to compare HIV disease progression between HIV-TB co-infected treated with standard TB therapy (control arm), versus co-infected patients treated with combined TB therapy plus ART (intervention arm) for 6 months. Control (n=38) and intervention (n=38) participants in this sub-study had additional immunologic assessments performed at study enrollment and every 3 months for a total of 12 months including: CD4 and CD8 T cell counts, markers of immune activation (CD38 and HLA-DR on CD4 and CD8 T cells), percentage of memory (CD45 RO+), naïve (CD45 RO-) and effector (CD62L-) CD4+ T cells, and T cell IFN-gamma production in response to the mitogen PHA and MTB and HIV antigens after a 7 day incubation. Sub-study participants were representative of all PART enrollees and were selected for sub-study enrollment based on the availability of laboratory results.

Flow Cytometric Analysis of Whole Blood

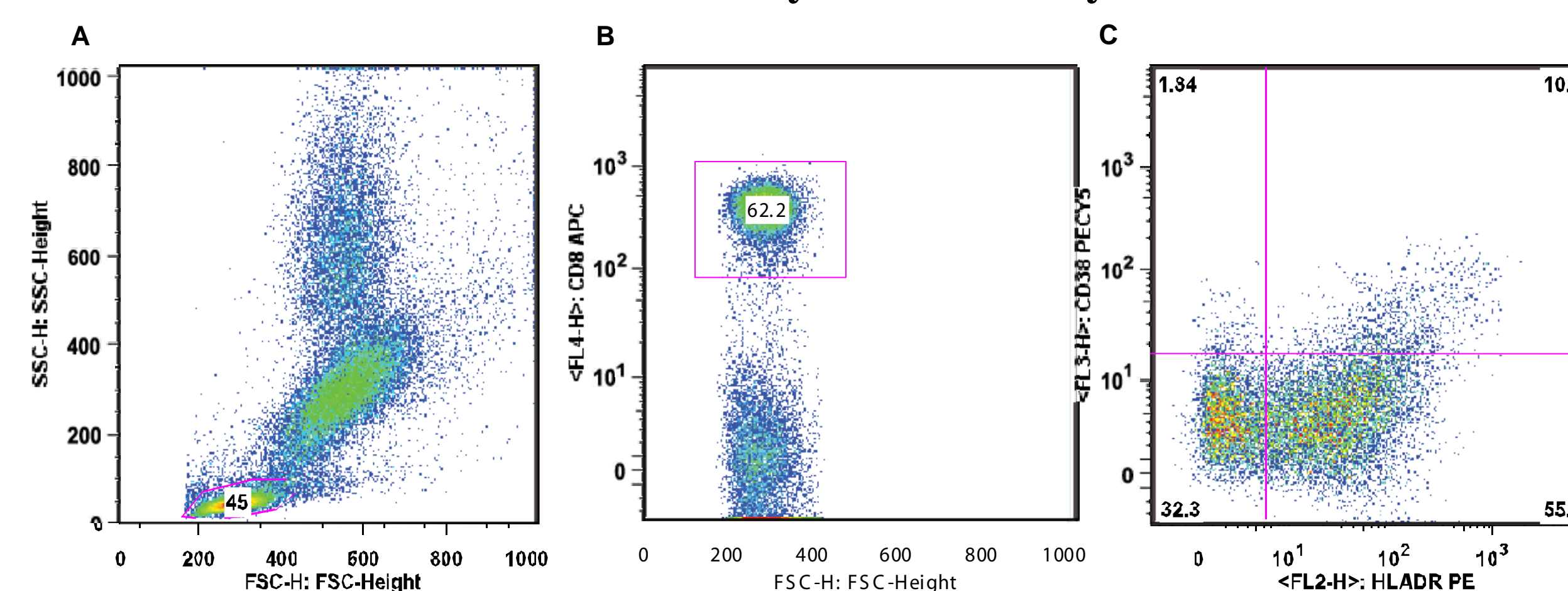


Figure 1. 4 color flow cytometry (Becton Dickinson FACSCalibur) was performed on whole blood. Gating on lymphocyte populations was based on forward and side scatter characteristics (A). Gate on CD8+ T cells (B). Delineation of HLA-DR+ and CD38+ cells (C). Isotype controls not shown.

RESULTS

Table I. Demographic and Baseline Clinical Characteristics of Study Participants

	Control arm (n=38)	Intervention arm (n=38)	p-value
Age	Median = 32 (range=23-48)	Median = 32 (range=19-54)	0.58
Gender	37% female	39% female	0.82
CD4 count	Median = 609.5 (range=374-1368)	Median = 532.5 (range=283-1415)	0.16
Viral Load	Log Median=4.4 (range=2.8-5.9)	Log median=4.5 (range=2.8-5.9)	0.84
Chest radiograph (extent of disease)	Normal/minimal: 18% Moderately advanced: 34% Far advanced: 47%	Normal/minimal: 21% Moderately advanced: 32% Far advanced: 47%	0.95
AFB Smear	Median grade = 3+	Median grade = 3+	0.25

Median HIV Viral Load during 12 months of Immunologic Follow-up

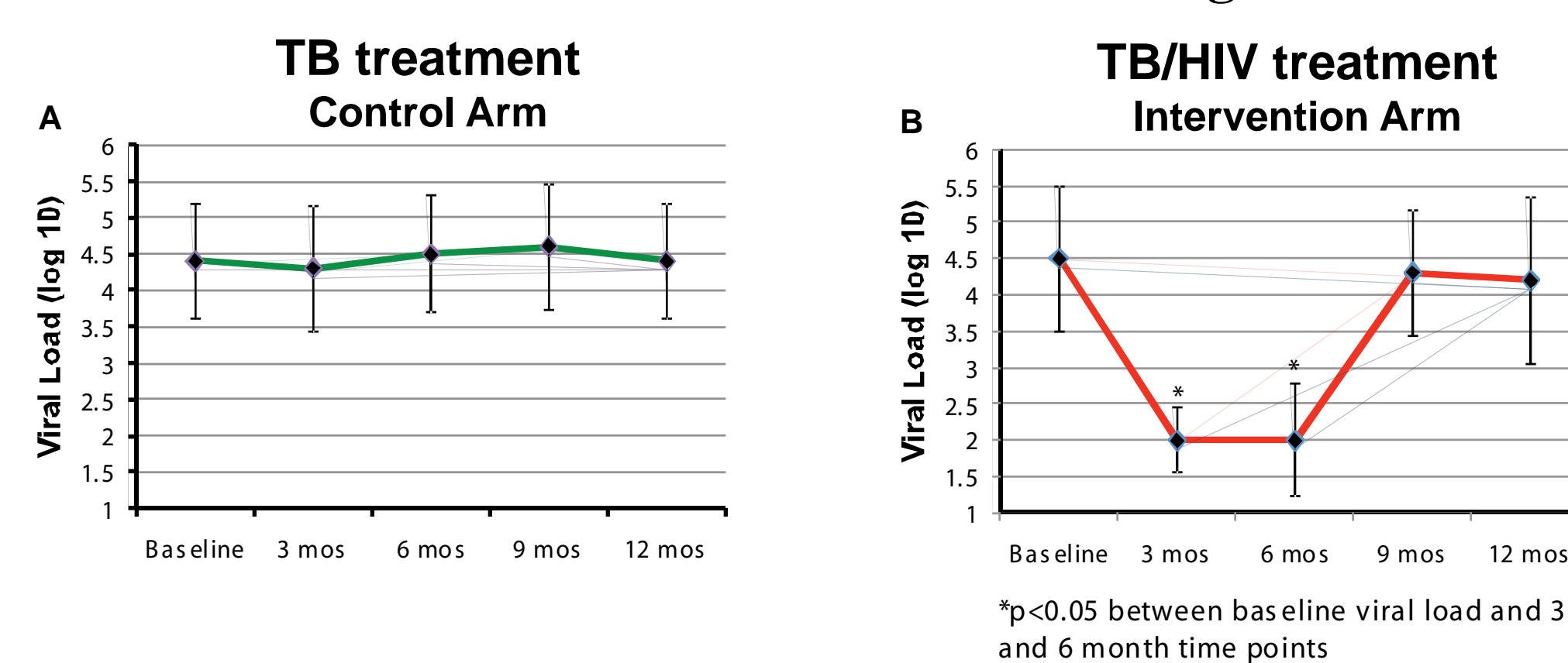


Figure 2. Median viral load was measured using Amplicor quantitative restriction transcriptase-polymerase chain reaction assay (Roche Amplicor 1.5) at study enrollment (baseline) and every three months through 12 months of study observation. The lower limit of detection of the assay was 400 copies/mm³. There were no significant changes in viral load in the control arm (A). A significant decrease in viral load was observed at 3 and 6 months in the intervention arm (B).

Table II. Total and CD4+ T cell subsets during 12 months of Immunologic Follow-up

	Control Arm	Intervention Arm	p-value*
Total CD4+ (absolute)			
Baseline	609.5	532.5	>0.05
6 months	540	584.5	>0.05
12 months	608.5	527	>0.05
Naïve (%) (CD4+/RO-/62L+)			
Baseline	47	44.1	>0.05
6 months	47.5	46.5	>0.05
12 months	49.4	48.3	>0.05
Memory (%) (CD4+/RO+/62L+)			
Baseline	38.5	39.3	>0.05
6 months	41.5	38.3	>0.05
12 months	35.2	36.3	>0.05
Effector (%) (CD4+/62L-)			
Baseline	12.8	14.8	>0.05
6 months	11.3	13.7	>0.05
12 months	15.3	11	>0.05

*Non-parametric, cross-sectional comparison between 2 arms

Sustained Improvement in T cell Effector Function with Treatment of TB/HIV Co-infection

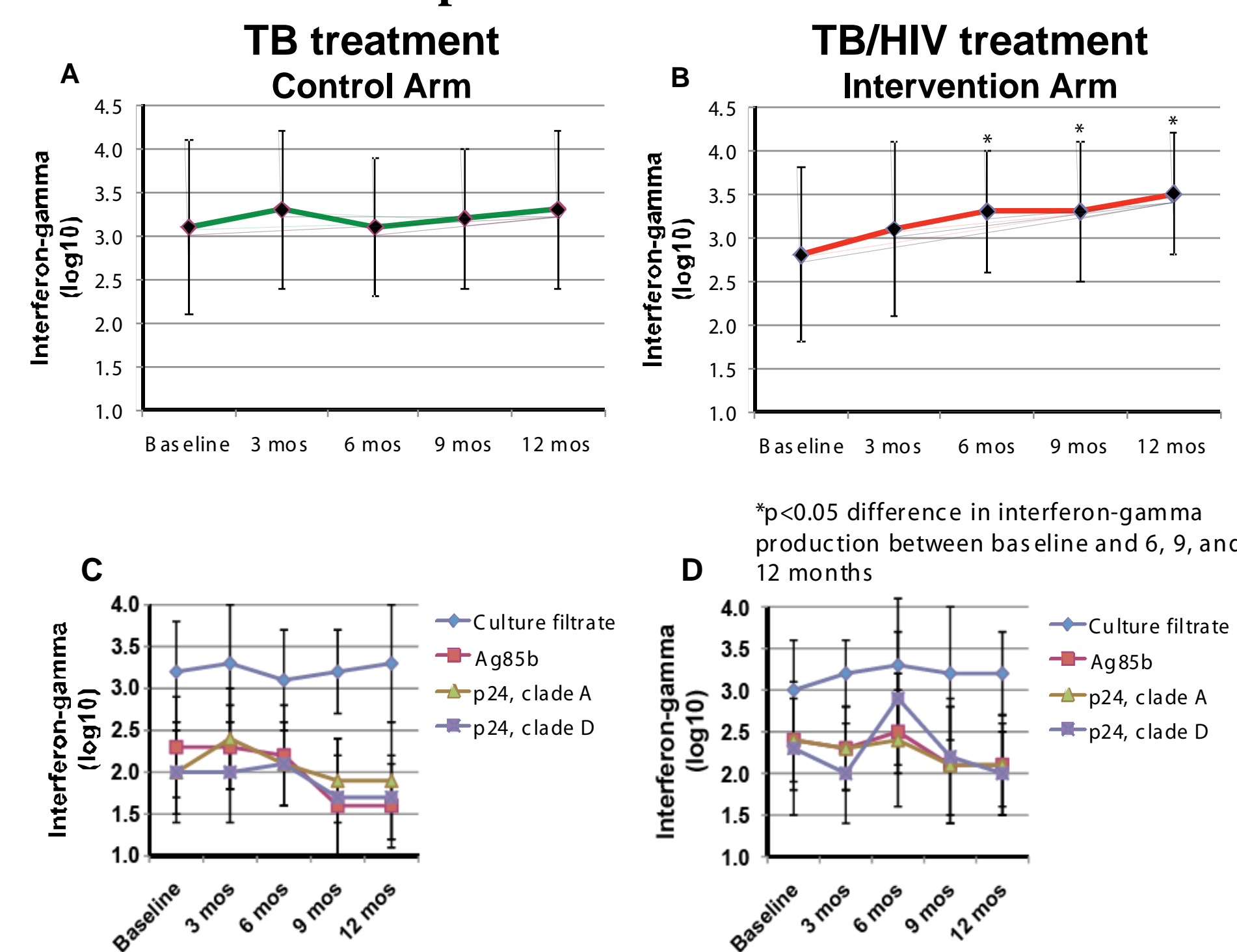


Figure 3. Whole blood interferon-gamma production was measured by ELISA following 7 day incubation with: PHA, MTB culture filtrate, Ag85b, p-24 protein from HIV clade-A and p-24 protein from HIV clade-D. Measurements were performed at study enrollment (baseline) and every 3 months through 12 months of study observation. There were no significant changes in interferon-gamma production in response to PHA or any of the MTB or HIV-specific antigens tested in the control arm (A, C). A significant increase in interferon-gamma production in response to PHA was observed in the intervention arm at 6, 9, and 12 months (B). There were no significant changes in interferon-gamma production in response to any of the MTB or HIV-specific antigens tested in the intervention arm (D).

Reduced Immune Activation with Treatment of TB alone and TB/HIV co-infection

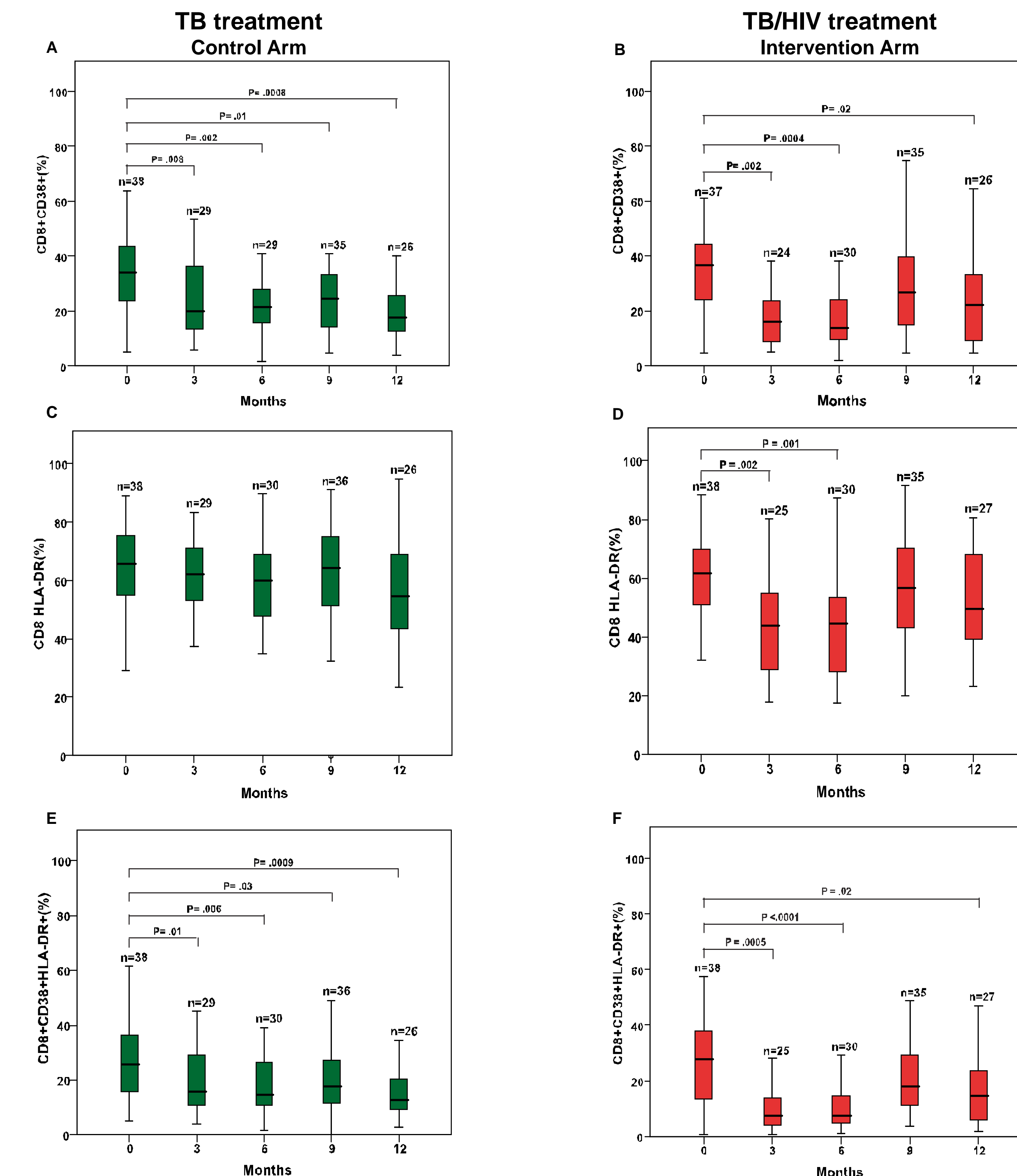


Figure 4. CD8 and HLA-DR expression on CD4+ and CD8+ T cells were measured by whole blood flow cytometry at study enrollment (baseline) and every three months through 12 months of observation. Among control subjects, there was a significant reduction in CD8+/CD38+ (A) and CD8+/CD38+/HLA-DR+ at all time points (E). CD8+/HLA-DR+ was not significantly decreased among controls (C). Control subjects demonstrated a significant reduction in CD4+/CD38+ and CD4+/HLA-DR+ at 12 months and a significant reduction in CD4+/CD38+/HLA-DR+ at 3, 6, and 12 months (CD4+ data not shown). Among intervention subjects, there was a significant reduction in CD8+/CD38+ (B) and CD8+/CD38+/HLA-DR+ at 3, 6, and 12 months (F). CD8+/HLA-DR+ was significantly decreased a 3 and 6 months in the intervention arm (D). Intervention subjects demonstrated a significant reduction in CD4+/CD38+, CD4+/HLA-DR+, and CD4+/CD38+/HLA-DR+ at all time points (CD4+ data not shown). At both 3 (p=0.01) and 6 (p=0.007) months of study observation, participants in the intervention arm had more significant reduction in CD8+/CD38+/HLA-DR+ than control participants (non-parametric, cross-sectional analysis).

CONCLUSIONS

•Among HIV-TB co-infected patients with preserved immune function, TB treatment alone (control arm) and concurrent TB/HIV treatment (intervention arm) produces sustained decreases in immune activation, and this reduction is most pronounced among intervention subjects while on therapy. Control subjects experience significant reduction in immune activation despite negligible reduction in HIV viral load. These findings suggest that treatment of TB alone allows for reduction of immune activation in co-infected patients.

•Treatment of TB alone and combined TB/HIV therapy increases the proportion of naïve CD4+ T cells.

•Concurrent TB/HIV treatment produces sustained increases in global T cell effector function as measured by IFN-gamma production in response to PHA. T cell responses to MTB and HIV specific proteins were not significantly improved in either study arm.

REFERENCES

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